

CLAIMS

1. Isolated photoprotein containing an amino acid sequence which:
 - a) is able to bind coelenterazine and calcium, producing
5 bioluminescence;
 - b) is identical by at least 90% to SEQ ID NO: 1 (Clytin);
 - c) in sequence alignment with SEQ ID NO: 1 (Clytin), presents one of the following single or multiple substitutions (the residue positions are referred to SEQ ID NO: 1):
 - 10 i) C₅₄→S;
 - ii) S₁₃₂→C;
 - iii) K₄₈→R, N₁₉₅→D;
 - iv) Q₆₈→R, A₉₀→V, T₁₈₄→I;
 - v) Y₈₂→F, K₁₁₀→N, F₁₂₅→L, S₁₄₉→R;
 - 15 vi) G₁₄₂→C;
 - vii) I₅₃→V, S₁₄₉→R;
 - viii) N₁₈→D, I₄₀→V, K₅₆→R;
 - ix) Gly₅₈→Glu, Asp₆₉→Val, Ala₇₀→Cys, Lys₇₆→Arg, Lys₇₇→Gly, Ile₇₈→Cys, Asp₈₁→Glu, Val₈₆→Ile, Glu₈₇→Ala, Ala₉₀→Gln,
20 Val₉₂→Leu, and Glu₉₇→Gln
2. The photoprotein of claim 1, containing an amino acid sequence identical by at least 95% to SEQ ID NO: 1.
3. The photoprotein of claim 2, containing an amino acid sequence identical by at least 98% to SEQ ID NO: 1.
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4. The photoprotein of claim 3, containing an amino acid sequence which is selected from the group consisting of SEQ ID NO: 2, 3, 4, 5, 6, 7, 8, 9, 10.
5. A photoprotein according to claims 1-4, wherein said amino acid

sequence is fused to a mitochondrial target sequence.

6. An isolated polynucleotide encoding a photoprotein according to claims

1-5.

7. The polynucleotide of claim 6, having the sequence of SEQ ID NO: 11,

5 12, 13, 14, 15, 16, 17, 18, 19.

8. An expression vector containing a polynucleotide according to anyone of claims 6-7.

9. A prokaryotic or eukaryotic host cell containing the vector of claim 8.

10. A mammalian host cell according to claim 9.

10 11. A method in vitro for detecting changes in intracellular calcium concentration which comprises:

a) providing a cell expressing a photoprotein according to claims 1-5;

b) contacting the cell with an agent stimulating calcium influx or calcium release from intracellular stores;

15 c) detecting the photoprotein bioluminescence.

12. A method of screening compounds modulating intracellular calcium concentration, which comprises:

a) providing a cell expressing a photoprotein of claims 1-5;

b) contacting the cell with the candidate compound;

20 c) detecting the bioluminescence of the photoprotein.

13. A method according to claims 11 or 12, which is carried out in a high-throughput format.

14. A method according to claim 13, which is carried out with a high throughput optical screening apparatus suited for multi-sample analysis.

25 15. The use of a photoprotein according to claims 1-5 as intracellular calcium indicator.

16. The use of a photoprotein according to claim 15 in a cell-based high throughput assay.

17. The use of a photoprotein according to claims 1-5 for the preparation of a diagnostic composition.